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MORPHOLOGY AND HISTOLOGY OF LOWER LIMB TENDONS AND LIGAMENTS IN PRIMATES

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

HENNING E. VON GIERKE
Director
Biodynamics and Bioengineering Division
Air Force Aerospace Medical Research Laboratory

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number):

A histological and morphological study of two tendons and two ligaments of the lower limb from three primate species is described ~~in this report.~~ Macro- and microscopic features were observed for the tendocalcaneus and flexor hallucis longus tendons and patellar and medial collateral ligaments of the rhesus monkey, baboon and chimpanzee. The histo- and morphological studies of lower limb ligaments and tendons in conjunction with mechanical studies provide information necessary to gain some insight into the mechanism of extremity injuries which have been observed in aircrewmen following ejection from high performance aircraft.

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SUMMARY

This report presents morphological and histological features of two tendons and two ligaments of the lower limb in three primate species. The research is being conducted in conjunction with the biomechanical analysis of lower limb ligaments and tendons in order to gain some insight into the mechanism of extremity injuries encountered during egress from high performance aircraft.

Specific histological studies were used to distinguish microscopic details, including fiber composition, alignment, structural morphology and proximal-distal change. Tendons and ligaments are remarkably similar in general compositions. Variations occur in size, amount of elastic fibers observed, and arrangement of loose connective tissue. The tissues selected for this study are found to sustain frequent injury in humans. Combining the morphological and histological data on these tissues with their biomechanical properties will greatly assist in the development of mathematical models of the musculoskeletal system for human injury research.

PREFACE

This study was conducted in the Department of Biomechanics, College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan 48824, under AF Contract No. F33615-79-C-0514. Dr. Robert Wm. Little, Professor and Chairman of the Department, was the Principal Investigator. Mr. Daniel J. Selke, the senior author, and Ms. Jane A. Walsh were also members of the Department of Biomechanics. This investigation, part of a three-year effort, supported Work Unit 7231-14-09, "Mechanical Stress on Soft Tissue Material Properties." Dr. Arnold R. Slonim of the Biodynamic Effects Branch, Biodynamics and Bioengineering Division, Air Force Aerospace Medical Research Laboratory, was the project scientist and contract monitor.

The authors gratefully acknowledge the valuable assistance of Mrs. Arvilla Bolley for her help in typing this report. The cooperation and assistance of Colonel A. R. Banknieder and his staff of the Veterinary Sciences Division, Air Force Aerospace Medical Research Laboratory, and of Dr. Frederick Coulston and Dr. Charles E. Graham, Director and Deputy Director, respectively, International Center of Environmental Safety of Albany Medical College, Holloman AFB, New Mexico, in providing primate cadaveric specimens vital to this study are very much appreciated.

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INTRODUCTION

Injuries to the extremities of crewmen during ejection from high speed aircraft have received much attention in recent years. For example, in a review of ejection cases from the F-4 aircraft from 1967 to 1977, Combs (3) reported that over 10% of non-combat ejections involved extremity injuries. Previous workers reported that under combat conditions, the extremity injury rate from windflail forces alone rose to 25%. Of the 95 extremity injuries for 43 F-4 ejectees, 61 were severe and 34, minimal; of the severe lower extremity ones, about half (10/22) were confined to the knee (3). The extremity injury rate statistics overall may be even greater if other high performance aircraft accident cases are similarly evaluated. Thus, there is a great need to study the major extremity tissues to understand the mechanism of their injury from mechanical forces. This study is in support of mechanical testing research on the lower limb ligaments and tendons of rhesus monkey, baboon and chimpanzee.

Tendons and ligaments are collagenous structures which form a major portion of the body's support system. Tendons are fibrous cords of connective tissue that are continuous with muscle fibers and serve to attach muscles to bones. Ligaments are also fibrous connective tissue which connects bones to other bones providing the necessary support and strength to the joints.

Most information on ligaments and tendons in the literature is anatomical or pathological in nature (1,13). Little is found to give a histological as well as morphological profile. Our study endeavors to describe the gross and histological features of two ligaments (patellar and medial collateral) and two tendons (tendo-calcaneus and flexor hallucis longus) of the lower limb from three primate species. Of particular interest are 1) microscopic composition of the tissues (collagen, elastin, ground substance), 2) structural changes in different regions (mid-area, proximal, distal), and 3) the general morphology of these structures. These observations provide a foundation for concurrent research in our laboratories.

METHODS AND MATERIALS

Selected tendons and ligaments were taken from rhesus monkey, baboon and chimpanzee carcasses, which were supplied by the Air Force. These animals were healthy and not previously used in experimentation. After death, the animals were chilled immediately and transported to our laboratories. Limbs were removed and stored at -5°C until further dissections could be carried out.

While frozen, the lower limbs were sectioned into knee regions and foot regions and stored separately. Prior to dissection the tissue was allowed to thaw, and the tendons and ligaments were then removed in a humid environment.

Dissection of the Knee:

Skin and extraneous tissues were removed to expose the ligaments of particular interest. The patellar ligament was removed first due to its accessibility. The sample was taken from the apex of the patella bone to the anterior tibia below the condyle, leaving a bone-to-bone attachment. The medial collateral ligament was dissected from the medial epicondyle of the femur to the medial tibia shaft (2,11). The bony attachments for both ligaments were sanded smooth to facilitate the utilization of mechanical testing grips.

Dissection of the Foot:

The plantar aspect of the foot was removed to expose the inner structures. The tendo-calcaneus tendon was dissected first and was freed of fascia from the gastrocnemius muscle to the calcaneum. It was then cut below the muscle and at the bone, leaving an area of bony attachment. The flexor hallucis longus muscle was exposed by removing the soleus muscle. Extraneous muscles and tendons were dissected out to free the flexor hallucis longus tendon. The tendon was cut before the osseo-fibrous tunnel and at the midpoint of the plantar exposure (2,11). After dissection, all tissues were stored moist at 4°C for not more than 24 hours, at which time mechanical testing was performed.

After testing, the tissues were fixed in 10% buffered formalin for several days followed by a post-fixation in Bouin's fluid for 24 hours. Samples then were sectioned in transverse and longitudinal planes and processed according to standard paraffin embedding methods. The tissue blocks were cut at 7μ thickness on a rotary microtome and stained. The stains routinely used were Hematoxylin-Eosin (10) for general morphology; Davenport's modification of Halmi's (4) for collagen and elastin; and Fraenkel's Orcein (9) for elastic fibers. Samples observed came from ligaments and tendons of both right and left limbs.

OBSERVATIONS

Tendo-calcaneus Tendon:

The tendo-calcaneus is a large broad tendon located in the posterior of the lower limb. It attaches the gastrocnemius muscle, which composes a large portion of the back of the leg, to the calcaneum (heel bone). Where the gastrocnemius fibers unite with the tendo-calcaneus, the soleus muscle tendon integrates into the main body of the tendo-calcaneus producing a "two-tendon" structure in the middle and proximal areas. At the tendon attachment to the calcaneus, there is a twisting and fanning out of the fibers, providing a broad area of insertion.

Microscopically, the tendo-calcaneus is composed of collagen with varying amounts of loose connective tissue and vasculature. Two segments are usually seen in the middle and proximal regions, but are not evident in the most distal sections (Fig. 1).

Figure 1: Tendo-calcaneus Tendon - Longitudinal Section - Baboon: The proximal area shows the soleus tendon (right) and the tendo-calcaneus (left) separated by loose connective tissue. At the far right is the loose connective tissue of the capsule.

Loose connective tissue and small blood vessels are seen between these segments. The collagen fiber bundles are parallel, slightly to moderately wavy in nature, and elastic fibers are evident in all areas (Fig. 2).

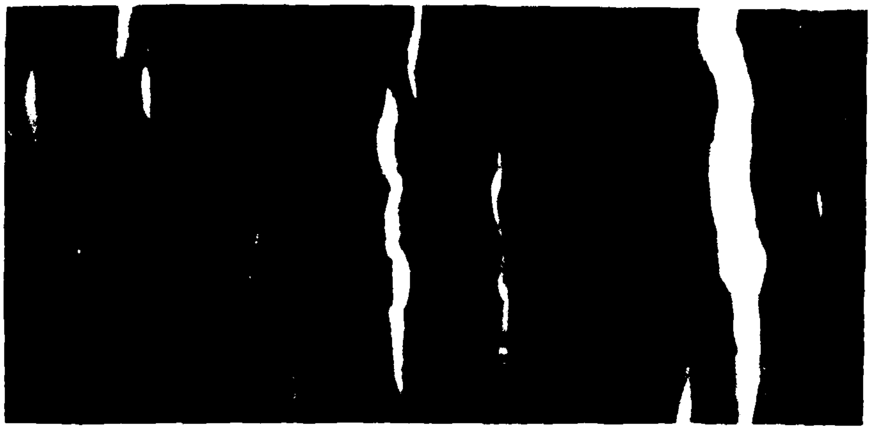

Figure 2: Tendo-calcaneus Tendon - Longitudinal Section - Baboon: Scattered elastic fibers in the slightly wavy collagen fiber bundles.

In areas of collagen-muscle union, muscle fibers are seen joining collagen fibers at approximately 30° angles (Figs. 3 & 4). The tendon capsule is composed of loose connective tissue with muscle fibers often seen on one side. This connective tissue varies from a compact layer at one surface to a broader area of loose connective tissue on the other.

Figure 3: Tendo-calcaneus Tendon - Longitudinal Section - Baboon: Photograph of the myotendinous junction at x100 power.

Figure 4: Tendo-calcaneus Tendon - Longitudinal Section - Baboon: Photograph of the myotendinous junction at x400 power from a section of Figure 3. Note the striations in the muscle fibers.

Flexor Hallucis Longus Tendon:

The flexor hallucis longus tendon is a rounded structure extending distally from the flexor hallucis longus muscle in the posterior of the lower leg. It continues through the osseo-fibrous tunnel, along the plantar aspect of the foot, through the fibrous digital sheath and attaches to the bottom of the distal phalange of the first toe.

At the microscopic level it is an ovoid structure made up of compact collagen fiber bundles with small amounts of loose connective tissue between the fascicles. Areas of vasculature are also found among the groups of bundles and muscle may be seen along the capsule surface on the anterior aspects. This anterior surface also tends to have loose connective tissue and blood vessles while the capsule on the posterior surface is very compact showing no loose connective tissue or vasculature (Figs. 5 & 6).

Figure 5: Flexor Hallucis Longus Tendon - Longitudinal Section - Rhesus Monkey: Capsular surface of the anterior aspect showing loose connective tissue and small blood vessels.

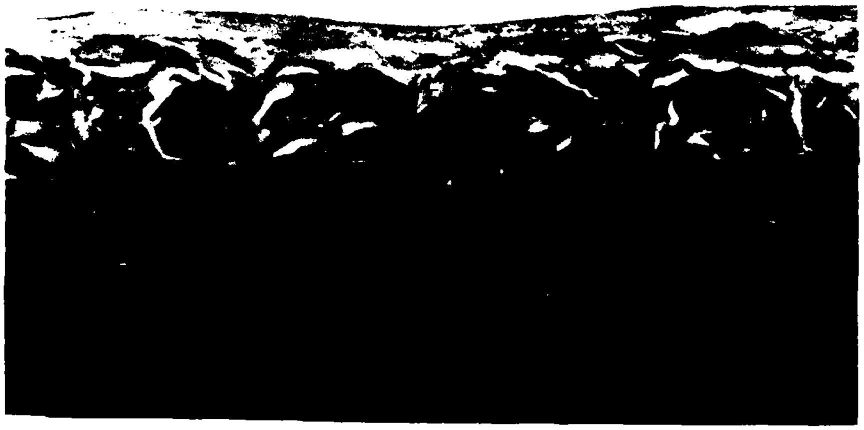

Figure 6: Flexor Hallucis Longus - Longitudinal Section - Rhesus Monkey: Capsular surface of the posterior aspect showing tightly bound connective tissue.

The fiber bundles are slightly to moderately wavy and parallel, with loose connective tissue between the bundles (Figs. 7 & 8). Elastic fibers are evident in all levels as well as in the surrounding loose connective tissue.

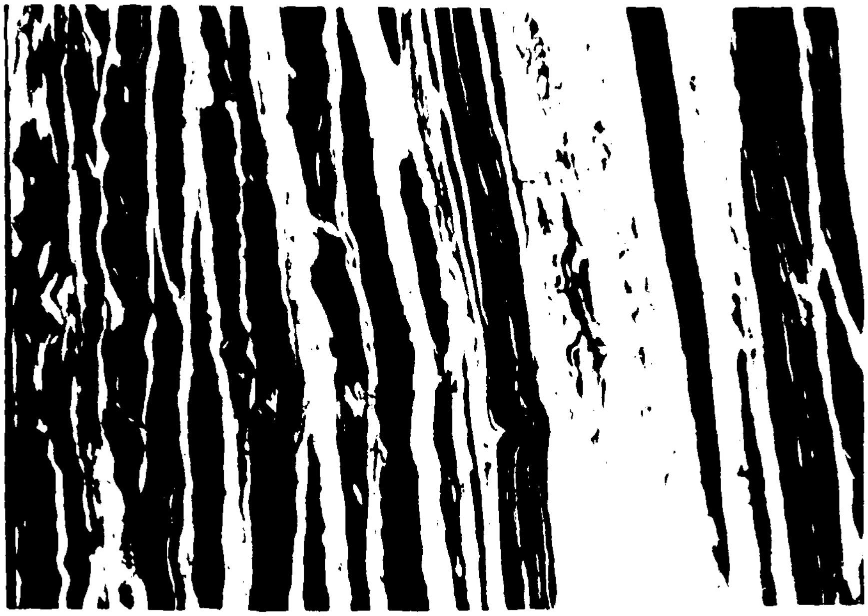

Figure 7: Flexor Hallucis Longus - Longitudinal Section - Chimpanzee: The tendon showing fiber bundles with loose connective tissue at x100 power.

Figure 8: Flexor Hallucis Longus - Longitudinal Section - Chimpanzee: The tendon showing fiber bundles with loose connective tissue at x400 power from a section of Figure 7.

Patellar Ligament:

The patellar is a broad, thick ligament situated on the anterior surface of the knee. It attaches to the tibia below the condyles and to the non-articulating area of the apex of the patella bone.

It is composed of compact collagen bundles with a broad area of loose connective tissue surrounding the joint capsule. Extensions of the capsular connective tissue invade the structure dividing the fascicles into segments. This is particularly noted at either end with less marked segmentation in the middle region. The fiber bundles are broad, parallel, and slightly wavy. Large areas of loose connective tissue and vasculature are seen between groups of bundles (Fig. 9). Elastic fibers are seen in all areas sampled.

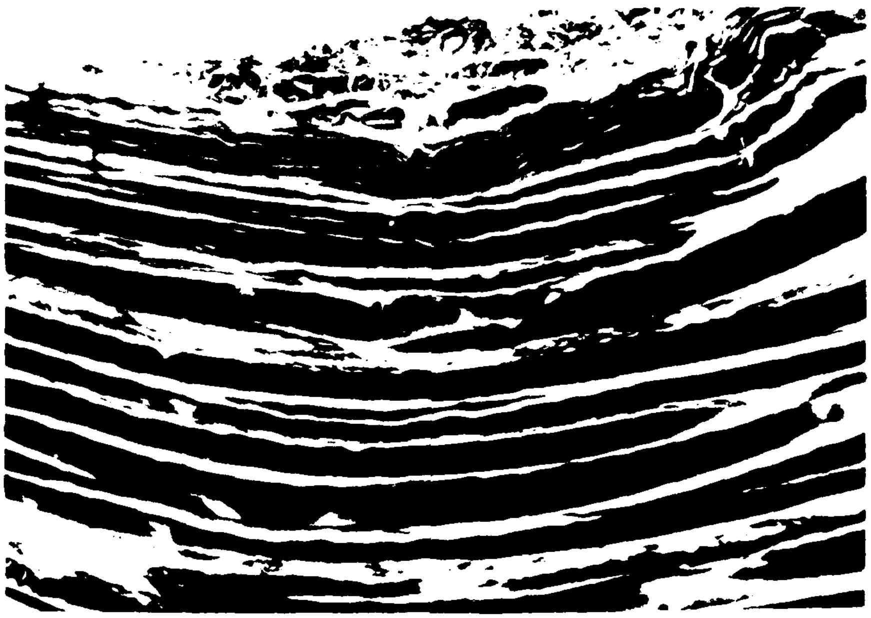

Figure 9: Patellar Ligament - Longitudinal Section - Chimpanzee: Parallel fiber bundles separated by loose connective tissue.

It is worthy to note that in the middle region on the outer edge of a longitudinal section of the patella, there exists a narrow band of parallel fibers that run perpendicular to the fiber bundles of the main ligament (Fig. 10). These fibers are moderately wavy and are separated from the rest of the ligament by a zone of loose connective tissue and blood vessels. Elastic fibers are seen in all areas, but are more prominent in the peripheral tissue.

Figure 10: Patellar Ligament - Longitudinal Section - Chimpanzee: A band of collagen fiber bundles is seen on the outer surface of the ligament. loose connective tissue separates this band from other ligamentous fibers.

Medial Collateral Ligament:

The medial collateral (also known as the tibial collateral) is a long, thin, flattened ligament. It attaches to the top of the medial epicondyle of the femur, transverses the medial aspect of the knee, and attaches to the medial tibial shaft.

Microscopically, it has an elongated cross-sectional structure composed of round fascicles with small amounts of loose connective and vascular tissue between them. A thick capsule surrounds the ligamentous structure. Longitudinally, the fiber bundles are compact, parallel, relatively acellular and moderately wavy. Loose connective tissue with some vasculature is observed in the capsular composition (Fig. 11). Elastic fibers are found in small numbers within the fiber bundles and in greater amounts in the ligament capsule.

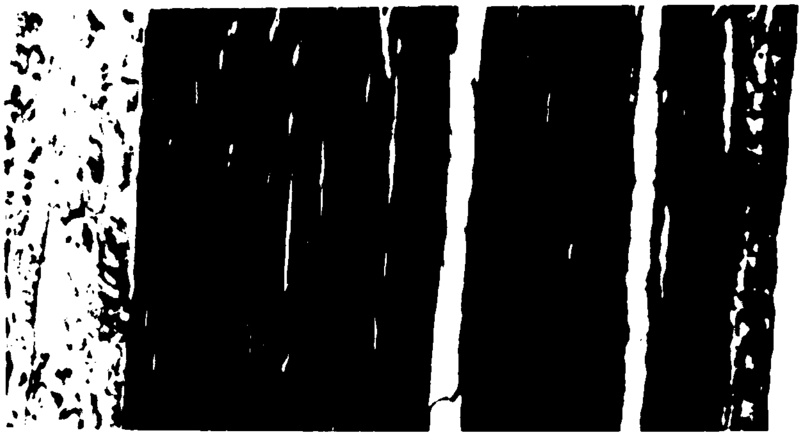

Figure 11: Medial Collateral Ligament - Longitudinal Section - Chimpanzee: Fiber bundles bounded on the posterior (left) by loose connective tissue and on the anterior (right) by compact connective tissue.

DISCUSSION

Tendons and ligaments have basic common histological characteristics. All are composed of parallel collagen fiber bundles held together by loose connective tissue. The bundles are comprised of fibers which are made up of fine fibrils in anintercellular matrix containing hyaluronic acid (5,8). Between these fiber bundles, fibroblasts are evident. The amount of elastic fibers in tendons and ligaments vary with the individual structures.

The tissues observed in this study exhibited general similarities and minor differences in cross-sectional areas which are tabulated in Tables 1-4. The cross-sectional areas were determined by placing metric graph paper under individual histological slides and "counting squares" while viewed under a microscope. For each ligament and tendon, no significant differences exist in the cross-sectional areas between the left and right limbs. Any variations in body size, weight and age of the individual specimens contributed to the differences in cross-sectional areas.

Geometrically the specimens were elongated and oval, with the exception of the flexor hallucis longus tendon which tended to be more rounded. Elastic fibers were visible in all the longitudinal sections as long branching fibers. They were difficult to detect in cross-sectional preparations due to their small diameters. Jack (7) indicates elastic fibers are absent in the human medial collateral ligaments but are found in the surrounding loose connective tissue. Our observations of the chimpanzee medial collateral showed elastic fibers in both the fiber bundles and the loose connective tissue.

The tissues used in the study are found to sustain frequent injury in humans; i.e. the medial collateral is most often affected by minor injuries or ruptures (12), the patellar exhibits frequent traumatic and congenital dislocations (13), and the tendo-calcaneus is subject to tears (6). It is therefore, desirable to have combined morphological, histological and mechanical data to further understand the properties of these structures. These studies have provided a foundation for further work with these tissues.

TABLE 1: TENDO-CALCANEUS TENDON

		LEFT FOOT			RIGHT FOOT		
Species	Region†	Representative†† Cross-Sectional Area, mm²	Total α Range, mm²	Total Cross-αα Sectional Area Average, mm²	Representative†† Cross-Sectional Area, mm²	Total α Range, mm²	Total Cross-αα Sectional Area Average, mm²
	U				11.50	11.0-12.0	
Rhesus	M	7.5	6.5-10.0		7.54	5.0-15.0	
	L				10.67	9.5-12.0	
				7.5			9.33
	U	19.83	19.0-21.0		16.61	14.0-22.5	
Baboon	M	18.35	16.0-21.5		14.0	13.0-15.0	
	L	23.8	22.0-28.0		17.6	12.0-27.0	
				20.07			16.71
	U				32.67	32.0-34.0	
Chimpanzee*	M	29.0	26.0-32.0		40.33	39.0-42.0	
	L	59.33	55.0-63.0		62.5	56.0-69.0	
				44.17			43.0

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near gastrocnemius muscle
M = middle area between upper and lower region
L = lower area near calcaneum.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

TABLE 2: FLEXOR HALLUCIS LONGUS TENDON

Species	Region†	LEFT FOOT: Representative†† Cross-Sectional Area, mm^2	LEFT FOOT: Total$^{\alpha}$ Range, mm^2	LEFT FOOT: Total Cross-Sectional Area Average, mm^2 $^{\alpha\alpha}$	RIGHT FOOT: Representative†† Cross-Sectional Area, mm^2	RIGHT FOOT: Total$^{\alpha}$ Range, mm^2	RIGHT FOOT: Total Cross-Sectional Area Average, mm^2 $^{\alpha\alpha}$
	U				9.75	9.5-10.0	
Rhesus	M	6.1	4.0- 8.0		6.81	4.0- 9.0	
	L				7.0	6.0- 8.0	
				6.1			7.12
	U	13.33	11.0-15.0		11.50	10.0-12.5	
Baboon	M	5.90	4.0- 8.0		7.50	7.0- 8.0	
	L	8.0	8.0		8.29	5.0-12.0	
				9.27			8.52
	U	27.97	27.0-29.0		13.2	9.0-12.0	
Chimpanzee*	M				14.33	13.0-16.0	
	L	25.2	25.0-26.0		16.0	11.0-20.0	
				26.55			14.27

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near fibrous distal sheath
M = middle area between upper and lower region
L = lower area near osseo-fibrous tunnel.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
$\alpha\alpha$Total cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

TABLE 3: PATELLAR LIGAMENT

Species	Region†	LEFT KNEE: Representative Cross-Sectional Area, mm^2 ††	LEFT KNEE: Total Range, mm^2 α	LEFT KNEE: Total Cross-Sectional Area Average, mm^2 αα	RIGHT KNEE: Representative Cross-Sectional Area, mm^2 ††	RIGHT KNEE: Total Range, mm^2 α	RIGHT KNEE: Total Cross-Sectional Area Average, mm^2 αα
	U	35.2	32-39		38.33	34-42	
Rhesus*	M	33.6	32-35		34.5	32-43	
	L	33.67	31-37		34.67	23-42	
				34.13			36.75
	U	43.25	33-47		41.0	37-45	
Baboon*	M	44.67	39-59		51.0	40-61	
	L	47.67	39-53		58.0	48-66	
				45.62			50.58
	U	110.0	100-116		81.0	80-82	
Chimpanzee*	M	77.5	77-78		80.0	80	
	L	109.33	102-115		100.0	83-110	
				101.63			87.75

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near apex of patella
M = middle area between upper and lower region
L = lower area near condyles of the tibial shaft.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

TABLE 4: MEDIAL COLLATERAL LIGAMENT

Species	Region†	LEFT KNEE			RIGHT KNEE		
		Representative†† Cross-Sectional Area, mm^2	Total$^{\alpha}$ Range, mm^2	Total Cross-$^{\alpha\alpha}$ Sectional Area Average, mm^2	Representative†† Cross-Sectional Area, mm^2	Total$^{\alpha}$ Range, mm^2	Total Cross-$^{\alpha\alpha}$ Sectional Area Average, mm^2
	U	5.75	4.0-8.0		8.29	6.5-10.0	
Rhesus*	M	4.88	3.0-5.0		4.75	3.0- 5.5	
	L	5.94	5.0-7.0		6.92	5.0- 8.0	
				5.52			6.74
	U	12.0	11.0-12.5		12.0	10.0-14.0	
Baboon*	M	9.83	8.0-12.0		4.3	3.0- 5.0	
	L	10.33	8.5-11.5		5.9	4.5- 7.0	
				10.47			8.15
	U	17.33	14.0-20.0		26.33	24.0-28.0	
Chimpanzee*	M	17.17	16.0-18.0		12.67	12.0-14.0	
	L	18.0	17.0-19.0		15.0	14.0-16.0	
				17.47			18.0

*Only one sample was tested for each limb from different specimens.
†Regions are defined as: U = upper area near epicondyle of femur,
M = middle area between upper and lower region
L = lower area near medial tibial shaft.
††Representative cross-sectional area is the total cross-sectional area for each of the individual regions.
αTotal range is the range of areas for each region.
ααTotal cross-sectional area average is the total number of upper + middle + lower areas divided by the number of samples measured.

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